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ABSTRACT:

PROBLEM TO BE SOLVED: To obtain a nature-derived oral antibacterial agent having excellent antibacterial action on intraoral bacteria causing caries, periodontal disease and widely applicable to various foods and drinks and oral products by effectively utilizing an extract of seeds or leaves of *Perilla frutescens crispa* or *Perilla ocimoides*.

SOLUTION: This oral antibacterial agent contains seeds or leaves of *Perilla frutescens crispa* or *Perilla ocimoides* as an active component. An alcohol extract of seeds or leaves of *Perilla frutescens crispa* or *Perilla ocimoides* is divided to ethyl acetate fraction and water-soluble fraction and the ethyl acetate fraction as the ethyl acetate layer may be used as the active component. As the seeds of *Perilla frutescens crispa* or *Perilla ocimoides*, defatted seeds of *Perilla frutescens crispa* or *Perilla ocimoides* may be used. The oral antibacterial agent contains luteolin, rosmarinic acid or their derivatives as active components. The foods and drinks or oral products contain this oral antibacterial agent.

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(54) ORAL ANTIBACTERIAL AGENT

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention is applied to products for the oral cavities, such as eating-and-drinking articles, such as gum, Kandy, juice, and GUMI, or kneading toothbrushing, mouth wash, and a prosthesis cleaning agent, concerning the antimicrobial agent for the oral cavities effective in prevention or the therapy of a caries, gum disease, etc.

[0002]

[Description of the Prior Art] Generally, it is known that gram-negative Bacilli, such as Streptococcus mutans and Porphyromonas gingivalis, will participate in a caries and gum disease, respectively. Streptococcus mutans forms a dental plaque in a tooth flank, and a caries disassembles various sugar, generates organic acids, such as a lactic acid and a citric acid, and happens by these organic acids' piling up continuously into a dental plaque, and eating enamel away. Moreover, gum disease is the chronic-inflammation nature disease of the bacteria origin, and a thing called the abnormalities of a host response to the increment in the parodontal pocket of periodontopathic bacteria, the bacterial invasion in the periodontium and a cell trauma, and infection has become the factor.

[0003] As a cure of such a caries and gum disease, antimicrobial agents, such as chloro HEKISHIJIN and cetyl pyridium chloride, are conventionally used for products for the oral cavities, such as mouth wash. If these antimicrobial agents are used so much, since we will be anxious about the effect affect the body, the present condition is that the addition is restricted. On the other hand, the extract of various vegetation is used for food, such as gum and a candy, as an antimicrobial agent aiming at prevention and the therapy of a caries and gum disease. For example, making into the active principle of an antimicrobial agent the epigallocatechin gallate which is one of the tea catechins is indicated by JP,7-25670,B.

[0004]

[Problem(s) to be Solved by the Invention] this invention persons -- Lamiaceae -- a beefsteak plant -- as a result of performing various trials about the extract component of the beefsteak plant and sesame belonging to a group, the outstanding antibacterial action to caries original **** and periodontopathic bacteria was found out to these seeds or leaf polyphenol. Moreover, it came to carry out the knowledge of the antibacterial action excellent in the luteolin and the loss marine acid which were refined from the seed polyphenol of a beefsteak plant or sesame existing.

[0005] The purpose of this invention is using effectively the seed of a beefsteak plant and sesame, or the extract component of a leaf, has the antibacterial action which was excellent to the dental bacteria leading to a caries and gum disease, and is to offer the antimicrobial agent for the oral cavities of the natural product origin which it can be large for various eating-and-drinking articles and products for the oral cavities, and can moreover be applied to them.

[0006]

[Means for Solving the Problem] The antimicrobial agent for the oral cavities of this invention for solving said technical problem is characterized by making the seed or leaf polyphenol of a beefsteak plant or sesame into an active principle. The seed of a beefsteak plant or sesame or the alcoholic extract of a leaf is distributed to ethyl acetate and water, and it is characterized by making into an active principle the ethyl-acetate distribution object obtained from this ethyl acetate layer. said beefsteak plant or a sesame seed -- cleaning -- a beefsteak plant -- it is characterized by using a seed or a cleaning sesame seed. It is characterized by making luteolin or a luteolin derivative into an active principle. It is characterized by making a loss marine acid or a loss marine acid derivative into an active principle. Moreover, it is characterized by the eating-and-drinking article of this invention coming to contain said antimicrobial agent for the oral cavities. Moreover, it is characterized by the product for the oral cavities of this invention coming to contain said antimicrobial agent for the oral cavities.

[0007] a beefsteak plant and sesame -- both -- Lamiaceae -- a beefsteak plant -- it is the vegetation of a group and has the relation of a variety mutually. a beefsteak plant is grown as savory herbs for many years -- having -- red -- a beefsteak plant and blue -- there is a beefsteak plant. Sesame is a Southeast Asia native therophyte and is an oil crop. The beefsteak plant is resembled at the whole, and it is a rectangle, and a stem is opposite in the leaf of an egg round shape, and attaches a white floret in summer. A seed is a little larger than a beefsteak plant, and is harvested in autumn. The oil which can be taken from a sesame seed is known as sesame oil or an oil of **, and is used for the raw material of edible and paint. Moreover, the oil cake is used as fertilizer and feed.

[0008] said seed polyphenol -- a beefsteak plant -- organic solvents, such as alcohol, an acetone, and ethyl acetate, can extract after crushing a seed or a sesame seed. The polyphenol component contained in the seed of a beefsteak plant and sesame is almost the same, and it is checked in investigation by artificers' high performance chromatography that both active principle can be extracted by the same approach. On the other hand, organic solvents, such as alcohol, an acetone, and ethyl acetate, can also extract said leaf polyphenol. It is checked that the leaf polyphenol component of a beefsteak plant and sesame almost contains the same component with high performance chromatography.

[0009] as the extract approach of said seed and leaf polyphenol -- a beefsteak plant -- it is good for alcohol to extract after grinding a seed or a sesame seed, and for ethyl acetate and water to distribute further. Thus, it is because the polyphenol concentration of an ethyl-acetate distribution object can be sharply raised by performing an alcoholic extract, and ethyl acetate / water distribution in order.

[0010] It is desirable to use ethanol for the alcohol used in order to extract said seed and leaf polyphenol. When ethanol is used, it is because it can be used even if it is external use and which an edible application at the same time an active principle is extracted efficiently. In addition, it is also possible to use a methanol, a butanol, etc. depending on an application.

[0011] About the water-solution concentration of the alcohol in the case of using alcohol as an extracting solvent, it is desirable to adjust to 70 - 85% (v/v). if the sampling volume of an active principle becomes it inadequate that it is under 70% (v/v) and it exceeds 85% (v/v) -- a beefsteak plant -- it is because it melts and becomes easy to take out the oil of a seed or a sesame seed into alcohol. In addition, an alcoholic extract is good to repeat by various concentration in order to raise the content of an active principle.

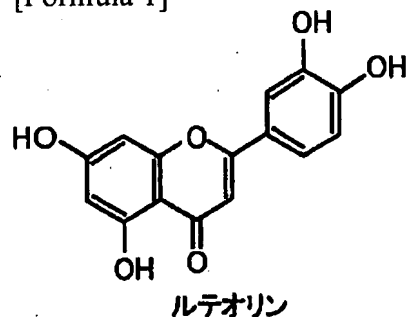
[0012] moreover, the active principle of the antimicrobial agent for the oral cavities by this invention -- a beefsteak plant -- it is desirable to extract from the cleaning object of a seed or a sesame seed. this -- an oily organic solvent -- a beefsteak plant -- it is because oil is removed from a seed and a sesame seed and polyphenol components, such as luteolin and a loss marine acid, are condensed in a cleaning object.

[0013] a beefsteak plant -- as an organic solvent for cleaning of a seed and a sesame seed, it is good to use a hexane. While being able to use a part for extracted oil as edible oil, it is because it is easy to use the extract from a cleaning object for a food material etc. Moreover, it is also possible to use other nonpolar solvents, without restricting to a hexane, when using the extract from a cleaning object for applications other than food.

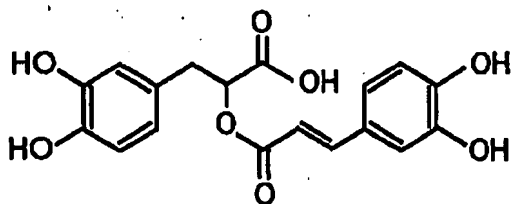
[0014] About luteolin and a loss marine acid, a simple substance can also be used as an active principle of an antimicrobial agent among the components contained in said seed or leaf polyphenol.

[0015] The structure expression of luteolin and a loss marine acid is as being shown below.

[Formula 1]



[Formula 2]



ロスマリン酸

[0016] It is known that luteolin generally exists as a glycoside in the vegetation (digitalis etc.) of Leguminosae. About the bioactive of luteolin, antioxidation activity, hyaluronidase inhibition activity, lipoxygenase inhibitory action (JP,10-298098,A), etc. are known conventionally. It can set to this invention, and luteolin can be extracted and refined also from these leaves besides the seed of sesame and a beefsteak plant. However, the luteolin contained in the leaf of sesame and a beefsteak plant is a minute amount, and it is desirable to use these seeds on the occasion of industrialization. It is also possible to obtain luteolin by the other approaches. For example, you may extract and refine from the vegetation of Leguminosae, the pericarp of a citrus system, etc.

[0017] A loss marine acid is a component characteristic of the Lamiaceae vegetation, and it is known that 1 - 2% of content is shown in a fresh leaf in the extensive group of Lamiaceae (pharmaceutical-sciences magazine vol.106, 1108-1111 1986 [P or J]). Anti-inflammation, an antiallergic operation, etc. are reported about the bioactive of a loss marine acid. It sets to this invention, and a loss marine acid is extracted and refined from the seed and leaf of sesame or a beefsteak plant, and also it can be obtained by the water alcoholic extract of the other Lamiaceae vegetation.

[0018] Said luteolin or Ross Marin in the form of these derivatives, i.e., a detail, moreover, sodium, The salts produced with metals, ammonium, etc., such as a potassium, magnesium, calcium, zinc, barium, and aluminum, Like the alkyl ester obtained by the reaction with alcohol, a fatty acid and alkyl halo GENAIDO, etc., ester and those salts, Moreover, the phosphorylation compound which introduced the phosphoric-acid radical, the sulfation compound which introduced the sulfuric-acid radical, Furthermore, the alkyl ether derivative by the reaction of the alkylene oxide which has the chain of extent of C2-C4 like ethylene glycol, propylene glycol, and a butylene glycol, It can use like glycidyl trialkylammonium halo GENAIDO in forms, such as the 4th class alkylamine derivative which produces the 4th class amine for a reaction with the substrate which it has in intramolecular, and its salts.

[0019] The antimicrobial agent for the oral cavities of this invention is confectionary and noodles (gum, a candy, a caramel, chocolate, Cookie, a snack, jelly, GUMI, hard candy, etc.) (a side), when adding and using for an eating-and-drinking article as a food material. Dairy products, such as Japanese noodles and a rahmen (milk, ice cream, yogurt, etc.), It can blend with common food including seasonings (bean paste, soy sauce, etc.), soup, and drinks (juice, coffee, tea, tea, a carbonated drink, sport drink, etc.), and health food (a tablet, capsule, etc.) and supplements (nutrition supplement drink etc.). Moreover, you may add to instant food. For example, an eating-and-drinking article can be made to contain easily what spray-dried or freeze-dried the antimicrobial agent for the oral cavities with powdered cellulose by making it powder, granulation, a making tablet, or a solution.

[0020] Moreover, the antimicrobial agent for the oral cavities of this invention may be blended with products for the oral cavities, such as kneading toothbrushing, powder toothbrushing, liquefied toothbrushing, and oiliness toothbrushing, mouth wash, a prosthesis cleaning agent, a gargle, and a gingival-massage cream. By blending the antimicrobial agent for the oral cavities with the product for the oral cavities used such every day, antibacterial effectiveness continues for a long period of time, and is useful to prevention of a cavity, gum disease, etc.

[0021]

[Effect of the Invention] As explained above, according to this invention, the antimicrobial agent for the oral cavities effective in prevention and the therapy of a caries and gum disease can be obtained by using the seed of a beefsteak plant or sesame, or the extract component of a leaf. Moreover, in order that the antimicrobial agent for the oral cavities of this invention may extract an active principle from the seed or leaf of the beefsteak plant known as a food raw material from the former, or sesame, it is safe and does not have the worries about a side effect etc. Moreover, it is possible to apply the antimicrobial agent for the oral cavities to various eating-and-drinking articles or products for the oral cavities widely, and prevention and the therapy of a caries and gum disease can be aimed at simply and effectively.

[0022]

[Embodiment of the Invention] Hereafter, the example of this invention is explained based on a drawing.

it is shown in [manufacture of antimicrobial agent for the oral cavities] drawing 1 -- as -- first -- a beefsteak plant --

what crushed the seed was flowed back by the hexane, and, subsequently the residue (cleaning object) was flowed back by ethanol 80% (v/v). Next, the hexane and 80% (v/v) methanol distributed the ethanol extract obtained by ethanol reflux 80% (v/v), and ethyl acetate and water distributed further after solvent distilling out of this methanol layer. The solvent of an ethyl acetate layer was distilled out after separating an ethyl acetate layer and a water layer, and the ethyl-acetate distribution object was obtained.

[0023] Subsequently, this ethyl-acetate distribution object was given to the silica gel column chromatography (chloroform: methanol =10:1), and the fraction 1 containing luteolin and the fraction 2 containing a loss marine acid were obtained. Then, the fraction 1 was suspended in the mixed solvent (chloroform: methanol =15:1), and luteolin was isolated from the insoluble fraction. Moreover, about the fraction 2, the loss marine acid was isolated with high performance chromatography.

[0024] As shown in drawing 1, what carried out solvent distilling out of the ethanol extract was made into the example 1, the ethyl acetate / water distribution of this ethanol extract were carried out, and the ethyl-acetate distribution object obtained from the ethyl acetate layer by carrying out solvent distilling out was made into the example 2. Moreover, each purification object of the luteolin and the loss marine acid which were isolated from the ethyl-acetate distribution object was made into the example 3 and the example 4, respectively. in addition, an example 1 - an example 4 -- a beefsteak plant -- although the extract obtained from the seed was used as the antimicrobial agent for the oral cavities, the antimicrobial agent for the oral cavities of the almost same presentation as an example 1 - an example 4 can be obtained also from a sesame seed by the same approach.

[0025] [Evaluation of an antibacterial action]

The streptococcus shown in the antibacterial action table 1 to an oral cavity streptococcus was inoculated into the brain heart infusion (BHI) liquid medium, it adjusted so that the 18 to 24-hour culture back might come at 37 degrees C and it might be come to 106-/ml with a physiological saline, and it considered as sample offering fungus liquid. The constant-rate smear of the sample offering fungus liquid was carried out to the BHI agar plate agar which carries out predetermined concentration content of the antimicrobial agent for the oral cavities of examples 1-4, and the minimum inhibitory concentration (Media Interface Connector) was judged after 24 to 36-hour culture at 37 degrees C. In addition, the antibacterial action was investigated according to conditions with the same said of the epigallocatechin gallate known as an antimicrobial agent of the tea-leaves origin as an example of a comparison. A result is shown in Table 1.

[0026]

[Table 1]

菌 種	実施例 1	実施例 2	実施例 3	実施例 4	比較例
<i>S. mutans</i> GS5	1600	100	100	1600	200
OMZ175	1600	100	100	1600	400
ATCC 25175	1600	200	100	>1600	800
ATCC 27352	400	50	50	1600	400
<i>S. sobrinus</i> OMZ176	1600	100	100	1600	400
<i>S. salivarius</i> ATCC 7073	>1600	400	800	>1600	200
AU 2613	>1600	200	200	>1600	800
<i>S. oralis</i> ATCC 35037	1600	50	100	1600	400
AU 1903	>1600	400	200	>1600	800
<i>S. mitior</i> ATCC 9811	>1600	200	200	>1600	800
<i>S. sanguis</i> ATCC 10556	>1600	200	400	1600	200
ATCC 10557	>1600	800	100	>1600	1600
AU 1023	>1600	800	400	>1600	1600

In addition, front Naka and ">1600" show that a minimum inhibitory concentration cannot judge under said conditions.

[0027] The result of Table 1 shows that an antibacterial action is shown to the mu factor wardrobe and SOBURINASU group which are said for the example 1 (ethanol extract), the example 2 (ethyl-acetate distribution object), the example 3 (luteolin purification object), and the example 4 (loss marine acid purification object) to participate in the caries

deeply. It was what shows the antibacterial action which was superior to the example of a comparison (epigallocatechin gallate) to almost all strains about the example 2 (ethyl-acetate distribution object) and especially the example 3 (luteolin purification object). Moreover, about *S.oralis*, *S.mitior*, and *S.sanguis*, participating in plaque formation is reported among Table 1. For this reason, about the example 2 (ethyl-acetate distribution object) and the example 3 (luteolin purification object), it was checked that it is effective also for control of the disease originating in formation of a plaque.

[0028] The periodontopathic bacteria shown in the antibacterial action table 2 to periodontopathic bacteria were inoculated into the GAM culture medium containing hemin (5microg/(ml)) and menadione (1microg/(ml)), and what adjusted *Porphyromonas gingivalis* to 108-/ml after 48-hour culture, and adjusted *Prevotella intermedia* and *Fusobacterium nucleatum* to 107-/ml at 37 degrees C was made into sample offering fungus liquid. The constant-rate smear of the sample offering fungus liquid was carried out to the GAM agar plate agar containing the antimicrobial agent for the oral cavities, and the hemin (5microg/(ml)) and menadione (1microg/(ml)) of the examples 1-4 adjusted to predetermined concentration, and the Media Interface Connector value was judged after 96-hour culture at 37 degrees C. In addition, the antibacterial action was investigated according to conditions with the same said of the epigallocatechin gallate known as an antimicrobial agent of the tea-leaves origin as an example of a comparison. A result is shown in Table 2.

[0029]

[Table 2]

菌 種	実施例 1	実施例 2	実施例 3	実施例 4	比較例
<i>P. gingivalis</i> BH18/10	800	100	25	800	50
RB24N-2	1600	100	25	800	100
OMZ314	800	50	12.5	800	200
W50	1600	50	25	800	200
6/28	1600	25	50	1600	100
381	1600	100	50	800	100
<i>P. intermedia</i> ATCC33563	>1600	>1600	200	>1600	>1600
<i>F. nucleatum</i> ATCC25586	>1600	800	100	1600	200

In addition, front Naka and ">1600" show that a minimum inhibitory concentration cannot judge under said conditions.

[0030] From the result of Table 2, the example 1 (ethanol extract), the example 2 (ethyl-acetate distribution object), the example 3 (luteolin purification object), and the example 4 (loss marine acid purification object) showed the antibacterial action to *Porphyromonas gingivalis* used as the main cause bacilli of gum disease. It was what shows the antibacterial action in which the example 2 (ethyl-acetate distribution object) and the example 3 (luteolin purification object) were extremely excellent to almost all strain especially.

[0031] When the antibacterial action to the strain of Table 1 and 2 is compared here, the antimicrobial activity of an example 2 (ethyl-acetate distribution object) is stronger than an example 1 (ethanol extract). This is considered because there are many polyphenol contents which are an active center by the example 2 compared with an example 1. Moreover, although an example 2 (ethyl-acetate distribution object) has a low luteolin content, the outstanding antimicrobial activity which is equal to an example 3 (luteolin purification object) is shown. This is considered that various kinds of polyphenol contained in an example 2 is raising antimicrobial activity by the interaction.

[0032] Chewing gum and a candy can be manufactured by the next formula using the antimicrobial agent for the oral cavities by [application to application 1: eating-and-drinking article] this invention. in addition, the inside of the following table -- "-- a beefsteak plant -- seed ethanol extract" dries the ethanol extract obtained by the same extraction condition as said example 1, and uses it as powder. moreover -- "-- a beefsteak plant -- seed ethyl-acetate distribution object" dries the ethyl-acetate distribution object obtained by the same extraction condition as said example 2, and uses it as powder.

[0033]

The example 1 of manufacture : Chewing gum Sugar 53.0wt(s)% The gum base 20.0 A glucose 10.0 A starch syrup 16.0 Perfume 0.5 a beefsteak plant -- seed ethanol extract 0.5 100.0wt% [0034]

The example 2 of manufacture : GUMI A restoration water candy 40.0wt(s)% Granulated sugar 20.0 Grape sugar 20.0

Gelatin 4.7 Water 9.68 Plum fruit juice 4.0 Plum flavor 0.6 Coloring matter 0.02 a beefsteak plant -- seed ethyl-acetate distribution object 1.0 100.0wt% [0035]

The example 3 of manufacture : A candy Sugar 50.0wt(s)% A starch syrup 33.0 Water 14.4 An organic acid 2.0 Perfume 0.2 a beefsteak plant -- seed ethyl-acetate distribution object 0.4 100.0wt% [0036] Tooth paste and mouth wash can be manufactured by the next formula using the antimicrobial agent for the oral cavities by [application to product for the application 2:oral cavities] this invention. in addition, the inside of the following table -- "-- a beefsteak plant -- seed ethanol extract" dries the ethanol extract obtained by the same extraction condition as said example 1, and uses it as powder. moreover -- "-- a beefsteak plant -- seed ethyl-acetate distribution object" dries the ethyl-acetate distribution object obtained by the same extraction condition as said example 2, and uses it as powder. [0037]

The example 4 of manufacture : Tooth paste A calcium carbonate 50.0wt(s)% A glycerol 20.0 The carrageenin 0.5 A carboxymethyl cellulose 1.0 RAURIUJI ethanol AMAIDO 1.0 Cane-sugar mono-laurate 2.0 Perfume 1.0 Saccharin 0.1 a beefsteak plant -- seed ethanol extract 0.4 Water 24.0 100.0wt% [0038]

The example 5 of manufacture : Tooth paste Dibasic calcium phosphate and 2 hydrate 50.0wt(s)% A glycerol 20.0 A carboxymethyl cellulose 2.0 Sodium lauryl sulfate 2.0 Perfume 1.0 Saccharin sodium 0.1 a beefsteak plant -- seed ethyl-acetate distribution object 0.5 Water 24.4 100.0wt% [0039]

The example 6 of manufacture : Mouth wash Ethanol 20.0wt(s)% Perfume 1.0 Lauryldiethanol amide 0.3 Mono-fluorophosphoric acid sodium 0.1 saccharin sodium 0.05 a beefsteak plant -- seed ethanol extract 1.0 Water 77.55 100.0wt% [0040]

The example 7 of manufacture : A prosthesis cleaning agent A mono-persulfuric acid hydrogen potassium 10.0wt(s)% Sodium perborate 70.0 Sodium tripolyphosphate 10.0 Sodium lauryl sulfate 1.0 enzyme 2.0 Coloring matter 0.02 Sodium carbonate 5.98 a beefsteak plant -- seed ethyl-acetate distribution object 1.0 100.0wt%

[Translation done.]